COMPARISON OF THE RATE OF MULTIPLICATION OF BACTERIA IN RAW MILK WITH THE RATE IN PASTEURIZED MILK*

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The object of the experimentation reported here was simply to compare the rate of increase of bacteria in raw milk with that in pasteurized milk. It is realized that only an investigation of wide scope could give any satisfactory information concerning the cause of certain divergencies in the rates of increase. It is possible, however, to gain some idea of the nature and the persistency of the variations from a series of comparative counts.

Aseptically drawn milk was obtained and 200-c.c. portions were measured out. One portion was pasteurized and the other was not. After pasteurization the treatment of the two samples was the same, the object being to eliminate every factor which might cause variation.

METHOD

Cows were selected which had a rather low udder content. At the morning's milking a quart of milk was aseptically drawn into a sterile bottle and immediately taken to the laboratory, where, after thorough shaking, 200 c.c. were placed in a sterile bottle and called Sample A. Another 200 c.c. were placed in another sterile bottle and called Sample B. The rest of the milk was labeled Sample C.

Sample A was then placed in ice water, Sample B was held for 30 minutes at 145 F., and Sample C was plated on 1% lactose agar in order to determine the number of bacteria in the milk as it came from the udder. After Sample B had been held for 30 minutes at 145 F., it was cooled to exactly 20 C. and at the same time Sample A was brought to 20 C., great care being taken that the bottles and the milk came to a constant 20 C. This was accomplished by the use of sterile thermometers and a water bath. To Sample A and to Sample B were then added the same number of bacteria from the same culture. After inoculation, Samples A and B were plated on 1% lactose agar at 2-hour intervals during 16 hours, 3 dilutions being used and 3 plates of each dilution, giving 9 plates from which to arrive at an average count of the number of bacteria in each sample at the end of each 2-hour period. The plates were incubated at 20 C. for 5 days and at 37 C. for 2 days and then counted.

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The organism added to the parallel samples of milk in Series 4, 5, and 6 was a typical Bacillus acidi-lactici isolated from Ericsson's butter culture. It had been propagated in the milk of cow 111R.F. for 3 days previous to inoculation into the samples in which the rates of growth were to be compared, the object being to eliminate, if possible, any preliminary decrease in numbers after inoculation, due to changed environment.

The method of determining how much of the milk culture to add to the samples in order to start them off with a definite number of organisms was as follows. Careful Breed counts were made of the milk

TABLE 1

DATA CONCERNING THE PLATING SERIES

Plating series	4	5	6
Date of sample of milk and of plating	12/20/15	12/20/15	3/22/15
Cow	111 L. F.	111 L. F.	191 R. F.
Amount of milk in sample, c.c.	200	200	200
Number of organisms in milk as it came from the udder	346	346	750
Organisms used for inoculation	B. acidi-lactici	B. acidi-lactici	B. acidi-lactici
Source of organism used	Ericsson's butter culture	Ericsson's butter culture	Ericsson's butter culture
Initial inoculation of raw sample	10,219 per c.c.	52,766 per c.c.	6850 per c.c.
Initial inoculation of pasteurized sample	10,028 per c.c.	47,366 per c.c.	7850 per c.c.
Incubation temperature, C.	20	20	20

culture, and from these counts it was calculated how many cubic centimeters of culture it was necessary to add to each sample in order to obtain the desired initial contamination.

In these three series the number of bacteria in the milk as it came from the udder was determined but not considered in the results of the platings, because the number of bacteria added to each sample was so large as compared with the number of udder organisms that it was judged the latter would have no appreciable effect on the results. And further it was observed that udder micrococci never appeared in

the Breed counts made for the purpose of maintaining proper dilutions in plating.

Into each of Series 8, 9, 10, 11, and 12 a chromogenic organism was inoculated, which because of the extreme color of its colony on agar could be easily distinguished on plates from udder organisms. By use of the Barber pipet[1] one bacterium from the selected chromogenic growth was added to a sample of raw milk and a second bacterium from the same growth was added to a sample of pasteurized milk. The bacterium had been taken with the pipet from an extremely dilute water suspension of a milk culture. In each case it had been accus-

TABLE 1—*Continued*

DATA CONCERNING THE PLATING SERIES

7	8	9	10	11	12
5/15/16	5/30/16	6/9/16	6/17/16	6/26/16	6/26/16
189 R. F.	150 R. F.	172 R. F.	213 R. F.	111 L. F.	209 R. F.
100	100	100	100	100	100
717	240	935	163	988	1191
Bacterium 222.233 2923 Pink	Bacterium 212.233 2324 Blue	Micrococcus 222.333 3623 Orange	Bacterium 222.233 2923 Pink	Bacterium 212.232 3532 Yellow	Bacterium 212.333 2612 Orange
Barn air	Utensils	Cow's mouth	Barn air	Utensils	Utensils
115 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.
130 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.	1 per 100 c.c.
20	20	20	20	20	20

tomed to growth in the milk of the special cow used in the experiment. After the plates made from the samples of milk inoculated with these chromogenic organisms had been incubated, it was easy to pick out the special chromogenic colonies and to follow the multiplication of the single chromogenic organism placed in the sample without confusing it with udder organisms which multiplied along with it.

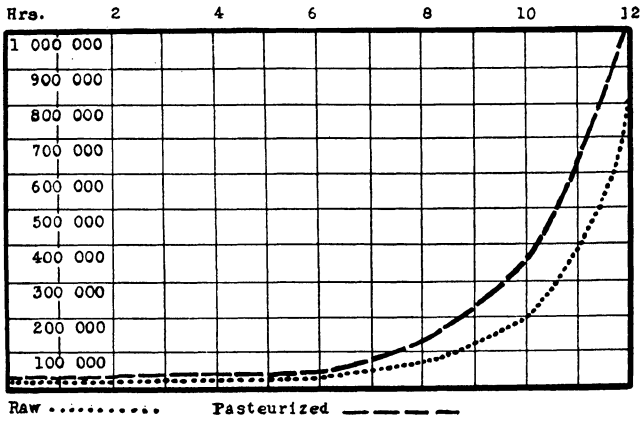
Graphs have not been made of Series 11 and 12 in the plating because of their similarity to the other series.

[1] Sc. Bull. Kansas Univ., No. 4, 1907.

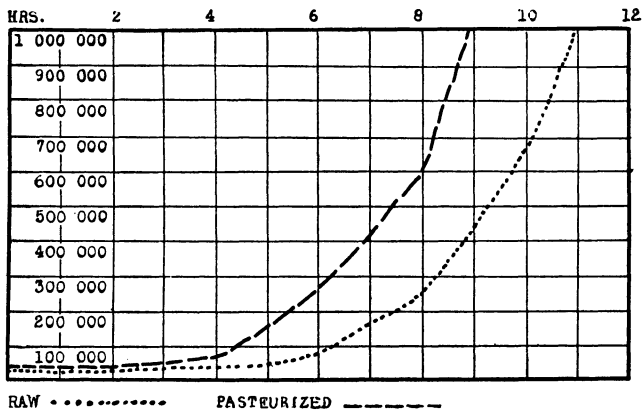
TABLE 2

COMPARISON OF THE RATE OF GROWTH OF B. ACIDI-LACTICI IN RAW MILK WITH THE RATE IN PASTEURIZED MILK

Hours After Inoculation	Average Number of Bacteria per c.c. in Raw Milk	Average Number of Bacteria per c.c. in Pasteurized Milk
	SERIES 4	
0	10,219	10,028
2	11,716	13,949
4	10,083	15,616
6	29,066	38,783
8	69,366	136,999
10	183,833	369,666
12	813,833	1,065,110
14	3,935,000	5,181,666
16	8,176,666	16,515,666
	SERIES 5	
0	52,766	47,366
2	47,283	46,066
4	41,749	63,483
6	80,800	247,833
8	247,999	597,166
10	672,499	1,711,666
12	1,671,666	3,121,666
14	9,293,333	14,263,533
16	16,566,666	34,216,666
	SERIES 6	
0	6,850	7,850
2	11,920	16,900
4	14,600	22,300
6	15,633	41,150
8	17,533	114,650
10	33,300	467,500
12	43,000	1,565,000
14	651,600	9,599,950
16	9,683,000	12,983,300
18	33,733,300	48,566,650
20	78,100,000	101,733,000
22	162,999,000	266,000,000



Series 4



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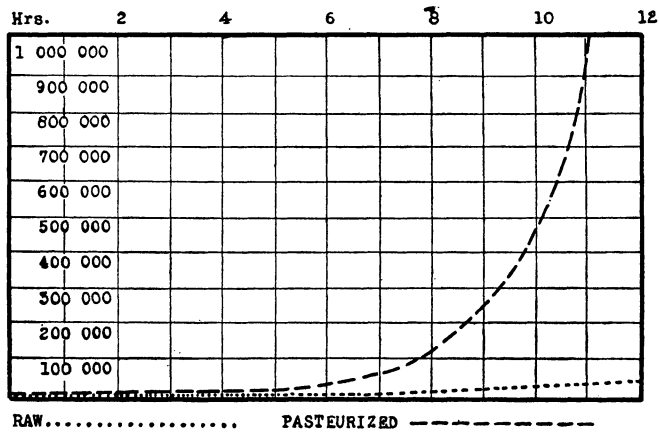

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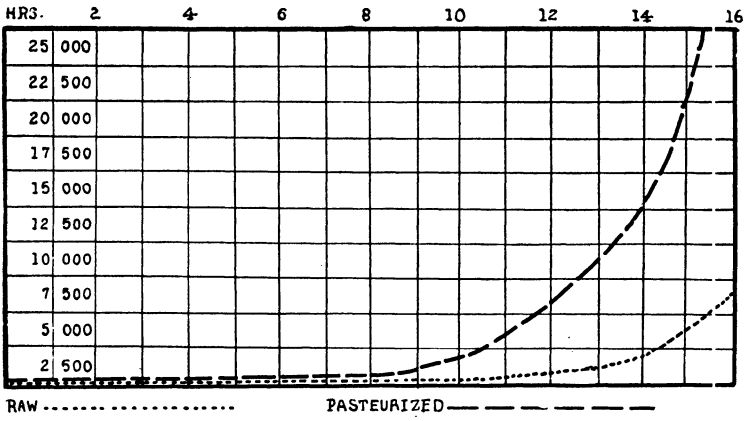

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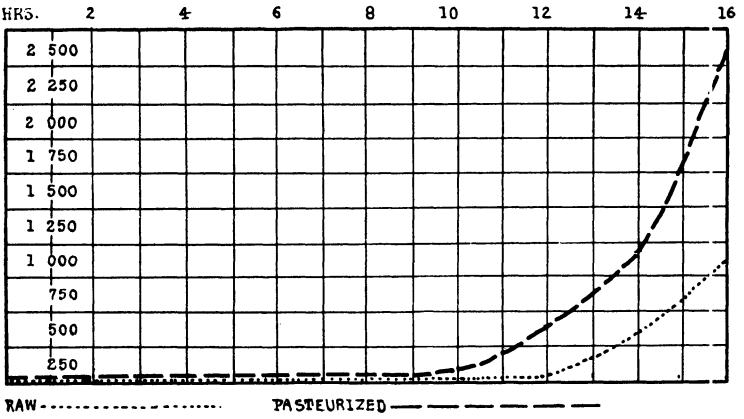

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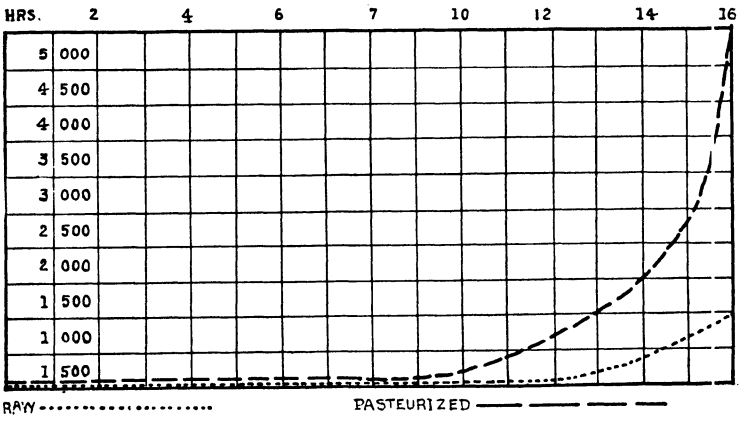
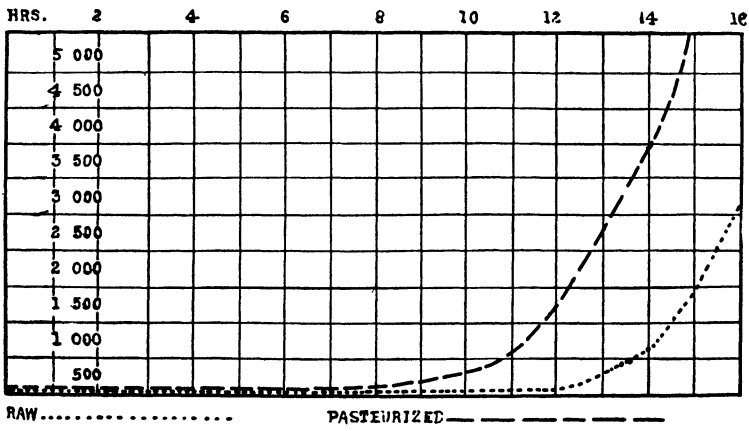


TABLE 3

COMPARISON OF RATE OF GROWTH OF CHROMOGENIC BACTERIA IN RAW MILK WITH THE RATE IN PASTEURIZED MILK

Hours after Inoculation	Average Number of Bacteria per c.c. in Raw Milk	Average Number of Bacteria per c.c. in Pasteurized Milk	Hours after Inoculation	Average Number of Bacteria per c.c. in Raw Milk	Average Number of Bacteria per c.c. in Pasteurized Milk
SERIES 7			SERIES 10		
0	1.15	1.3	0	0	0
2	13	18	2	0	0
4	77	97	4	0	0
6	149	353	6	2	11
8	173	536	8	14	80
10	295	1929	10	45	326
12	778	6083	12	165	1215
14	1687	15,666	14	669	3461
16	6566	32,574	16	2738	7003
SERIES 8			SERIES 11		
0	0	0	0	0	0
2	0	0	2	0	0
4	0	1	4	0	1
6	4	10	6	7	3
8	12	27	8	15	13
10	23	63	10	33	94
12	150	368	12	100	536
14	334	900	14	418	1820
16	872	2508	16	1120	3558
SERIES 9			SERIES 12		
0	0	0	0	0	0
2	0	0	2	0	0
4	0	0	4	0	0
6	1	6	6	0	9
8	9	28	8	8	25
10	21	104	10	30	206
12	78	813	12	181	862
14	357	1469	14	615	1756
16	2979	5167	16	1658	6258



Series 10

CONCLUSIONS

Raw milk as compared with pasteurized milk exerts a powerful suppressing influence on the multiplication of certain bacteria.

When Bacillus lactici-acidi is accustomed to the milk of a certain cow, apparently no killing off of this organism takes place in freshly drawn milk.

When a single cell of certain pronouncedly chromogenic kinds of bacteria is added to fresh milk, the organism is found plentifully in the milk after 16 hours at 20 C., the injurious action of freshly drawn milk not being sufficiently intense to kill the one bacterial cell.

After pasteurization the organisms which remain in the milk and those which are able to get into the milk find conditions more favorable for their rapid multiplication than before pasteurization.